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Epidemiological analysis of *Neisseria gonorrhoeae* in the Federal Republic of Germany by auxotyping and serological classification using monoclonal antibodies

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SUMMARY We evaluated a new serological classification system for *Neisseria gonorrhoeae* based on monoclonal antibodies directed against epitopes on the outer membrane protein I, in conjunction with auxotyping, to analyse gonococci from two cities in the Federal Republic of Germany. Isolates of *N gonorrhoeae* were collected during 1976-8 and 1980-2 in Lübeck, and during 1980-2 in Heidelberg. Between the two study periods in Lübeck, we observed an appreciable decrease in strains of the auxotype that requires arginine, hypoxanthine, and uracil (AHU^-) and with serovar class PrIA-1 and the emergence of strains with the proline requiring auxotype and PrIB-1 serovar class. Serovar PrIA-1 accounted for 89 (97%) of 92 strains with the AHU^- auxotype as opposed to 12 (4%) of 297 strains with other auxotypes ($p<0{\cdot}0001$). Disseminated gonococcal infection was associated with $AHU^-/PrIA^-1$ strains. Penicillinase producing *N gonorrhoeae* (PPNG) strains belonged to eight different auxotype and serovar classes, which indicated that different strains had been imported. Classification of strains of *N gonorrhoeae* by auxotype and serovar class permits analysis of temporal changes in gonococcal populations, and of migrations of gonococci between different geographical areas. Typing *N gonorrhoeae*, together with assessing antibiotic susceptibilities, may prove useful for further studies of the epidemiology and control of gonorrhoea.

Introduction

Since 1973, when Catlin introduced auxotyping for *Neisseria gonorrhoeae*,[1] clinical isolates have been identified by their growth requirements on chemically defined media lacking different amino acids or pyrimidines. In 1980 Sandström and Danielsson described the serological classification of *N gonorrhoeae* by a coagglutination method.[2] Tests performed with absorbed rabbit antisera permitted the classification of strains of *N gonorrhoeae* into three serogroups termed WI, WII, and WIII. Serogroup specificity is based on the antigenic heterogeneity of the protein I molecule in the gonococcal outer membrane.[3] Peptide mapping studies have shown two protein I molecules, designated PrIA and PrIB. WI serogroups possess PrIA, whereas WII and WIII serogroups possess PrIB.[4] In 1982 Tam *et al* introduced monoclonal antibodies directed against PrIA and PrIB for use in the coagglutination test.[5] These and additional monoclonal antibodies specific against PrIA are capable of differentiating between gonococci with a large number of reaction patterns, called serovars.[6] A nomenclature has been proposed for these serovars,[6] and auxotype and serovar classification has been used to describe gonococcal isolates.[6]

In the study published here we used auxotype and serovar classification to examine the populations of gonococcal strains in two different geographical areas of the Federal Republic of Germany (FRG): Lübeck, a Baltic Sea port, and Heidelberg, a southern inland city. Furthermore we assessed correlations between auxotypes and serovars for *N gonorrhoeae* isolates from the FRG.

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Materials and methods

In Lübeck we collected 84 *N gonorrhoeae* isolates during 1976-8 and 94 in 1980-2. In Heidelberg we collected 211 isolates from 1980 to the summer of 1982. All isolates were deposited in the culture collection of the Neisseria Reference Laboratory and stored at -70°C in a mixture of equal amounts of trypticase soy broth and heat inactivated horse serum free from γ globulin.

We performed auxotyping on two different chemically defined media: Catlin's defined agar medium for growing *Neisseria* spp,[1] and the modified defined medium for *N gonorrhoeae* described by Morse and Bartenstein.[7] We modified the latter medium by decreasing the uracil concentration to one sixth of the original concentration; this improved the growth of strains that required arginine, hypoxanthine, and uracil (AHU$^-$). All strains were tested for their requirement for proline, arginine, hypoxanthine, uracil, and methionine. All strains required cystine and cysteine for growth. Gonococci of known auxotypes were included in each auxotyping run.

We performed serological classification by a co-agglutination test as described previouly.[2 6] We used six monoclonal antibody reagents (4A12, 4G5, 2F12, 6D9, 5G9, and 5D1) specific against PrIA and six (3C8, 1F5, 2D6, 2G2, 2D4, and 2H1) specific against PrIB. Each coagglutination reaction was performed by mixing one drop of a suspension of boiled gonococci with one drop of a suspension of sensitised staphylococci, rotating the mixture for two minutes, and observing it for agglutination under oblique transmitted light. We used the serovar nomenclature of Knapp *et al.*[6]

We used Fisher's exact test to assess the statistical significance of our results.

TABLE I *Distribution of auxotypes of Neisseria gonorrhoeae from Lübeck in 1976-8 and 1980-2 and from Heidelberg in 1980-2*

	No (%) of isolates from:		
Nutritional requirements	*Lübeck 1976-8 (n=84)*	*Lübeck 1980-2 (n=94)*	*Heidelberg 1980-2 (n=211)*
AHU$^-$	46(55)*	17(18)*	29(14)
Prototrophic	30(36)	48(51)	112(53)
Pro$^-$	2 (2)*	26(28)*	48(23)
Arg$^-$	1 (1)	2 (2)	6 (3)
Pro-Hyx$^-$	0 (0)	0 (0)	11 (5)
PAU$^-$	5 (6)	(1)	5 (2)

*=p<0·0001 comparing Lübeck 1976-8 with Lübeck 1980-2.
AHU$^-$ = arginine, hypoxanthine, and uracil requiring; Pro$^-$ = proline requiring; Arg$^-$ = arginine requiring; Pro$^-$Hyx$^-$ = proline and hypoxanthine requiring; PAU$^-$ = proline, arginine and uracil requiring.

Results

TEMPORAL SHIFT IN DISTRIBUTION OF AUXOTYPES IN LÜBECK

Table I shows the distribution of auxotypes by city and time. The AHU$^-$ auxotype was found in 46 (55%) of 84 strains from Lübeck in 1976-8. In contrast, in 1980-2, the AHU$^-$ auxotype was found in only 17 (18%) of 94 strains from Lübeck (p<0·0001) and 29 (14%) of 211 strains from Heidelberg. At the same time as the decrease of AHU$^-$ auxotypes in Lübeck, strains with the proline requiring (Pro$^-$) auxotype increased from two (2%) out of 84 to 26 (28%) out of 94 (p<0·0001). The distribution of other auxotypes during the two periods in Lübeck and during 1980-2 in Heidelberg was similar.

RELATION OF AUXOTYPES TO SEROVARS

Table II shows the association between auxotypes and serovars in our study population. Nine PrIA and 14 PrIB serovars were identified among all strains from Lübeck and Heidelberg. The figure shows the reaction pattern of these serovars.

As Table II shows, there were several associations between auxotypes and serovars in the 389 strains. Though the requirement for proline was associated with six PrIA and nine PrIB serovars, it was associated most strongly with serovar PrIB-1. Auxotypes that required proline, arginine, and uracil (PAU$^-$) were associated with the closely related serovars, PrIB-2 and PrIB-16.

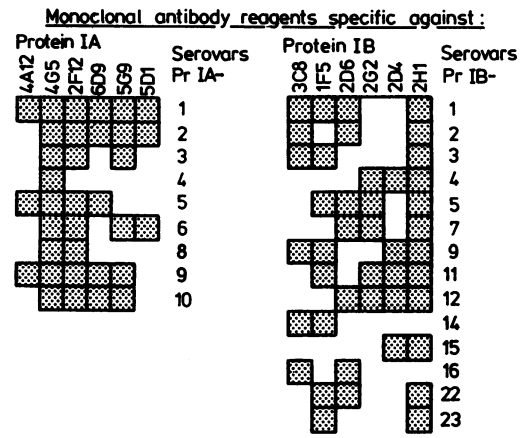

FIGURE *Neisseria gonorrhoeae protein IA (PrIA) and IB (PrIB) serovars detected among 389 strains isolated during 1976-8 and 1980-2 in Lübeck and during 1980-2 in Heidelberg in the Federal Republic of Germany. Shaded boxes represent positive reactions with the monoclonal antibody reagents listed above the columns. Serovars were defined by the patterns of reactions with either PrIA or PrIB monoclonal antibody reagents, and the serovar nomenclature of Knapp et al[6] was used.*

TABLE II *Distribution of six auxotypes and nine PrIA and 14 PrIB serovars in 389 strains of Neisseria gonorrhoeae from Lübeck and Heidelberg*

	No (%) of strains with auxotypes:					
Serovar	*AHU⁻ (n=92)*	*Prototrophic (n=190)*	*Pro⁻ (n=76)*	*Arg⁻ (n=9)*	*Pro⁻Hyx⁻ (n=11)*	*PAU⁻ (n=11)*
PrIA-1	89(97)*	10 (5)	2 (3)			
PrIA-2		1 (0·5)				
PrIA-3		1 (0·5)	2 (3)			
PrIA-4		5 (3)	1 (1)			
PrIA-5	2 (2)	1 (0·5)				
PrIA-6	1 (1)	5 (3)	11 (15)	2 (22)		
PrIA-8		2 (1)	2 (3)			
PrIA-9		1 (0·5)	1 (1)			
PrIA-10		1 (0·5)				
Total	92	27	19	2	0	0
PrIB-1		36 (19)	27 (36)*	2(22)	1 (9)	2 (18)
PrIB-2		13 (7)	7 (9)			5 (45)*
PrIB-3		73 (38)	9 (12)	5 (56)	10 (91)	
PrIB-4		12 (6)	4 (5)			
PrIB-5		15 (8)	2 (3)			
PrIB-7			3 (4)			
PrIB-9		1 (0·5)	1 (1)			
PrIB-11		2 (1)	3 (4)			
PrIB-12		1 (0·5)				
PrIB-14		1 (0·5)	1 (1)			
PrIB-15		1 (0·5)				
PrIB-16		1 (0·5)				4 (36)*
PrIB-22		5 (3)				
PrIB-23		2 (1)				
Total	0	163	57	7	11	11

*= $p<0·0001$ for association of auxotype with serovar.
AHU⁻ = arginine, hypoxanthine, and uracil requiring; Pro⁻ = proline requiring; Arg⁻ = arginine requiring; Pro⁻Hyx⁻ = proline and hypoxanthine requiring; PAU⁻ = proline, arginine and uracil requiring.

The strongest correlation between auxotype and serovar was between the AHU⁻ auxotype and the PrIA-1 serovar, which was characterised by a reaction with all six PrIA monoclonal antibodies. Serovar PrIA-1 was found in 89 (97%) out of 92 strains with AHU⁻ auxotypes and 12 (4%) out of 297 strains with other auxotypes.

EMERGENCE OF A NEW STRAIN OF *N GONORRHOEAE* IN LÜBECK IN 1980-2

Dual classification by auxotype and serovar showed that the decrease of AHU⁻ auxotypes was due to a decrease in AHU⁻/PrIA-1 isolates. The increase of strains with Pro⁻ auxotypes was mainly attributable to the appearance of Pro⁻/PrIB-1 isolates; these isolates were not detected in Lübeck in 1976-8, but by 1980-2 represented 14 (54%) out of 26 Pro⁻ isolates from Lübeck ($p<0·05$), and 13 (29%) out of 47 Pro⁻ isolates from Heidelberg.

To assess whether the decrease of AHU⁻/PrIA-1 isolates and the introduction of Pro⁻/PrIB-1 isolates into the gonococcal population of Lübeck were associated with specific groups of patients, we separately analysed isolates from men with uncomplicated urethritis and from prostitutes and non-prostitutes with uncomplicated cervical infection. In men and prostitutes, we observed a significant ($p<0·05$) decrease in the population of isolates belonging to the AHU⁻/PrIA-1 class between 1976-8 and 1980-2 (table III). The new Pro⁻/PrIB-1 strain showed a significant ($p<0·05$) increase only among strains causing uncomplicated urethritis in men (table IV).

DISSEMINATED GONOCOCCAL INFECTION ASSOCIATED WITH AHU⁻/PrIA⁻1 ISOLATES

The strains available for study included seven *N gonorrhoeae* strains isolated from patients with disseminated gonococcal infection. Five strains were isolated in Lübeck in 1976-8, one in Lübeck in 1980-2, and one in Heidelberg in 1980-2. Six (86%) out of the seven isolates were AHU⁻/PrIA-1 strains, and one was AHU⁻/PrIA-5. We matched the seven isolates with seven obtained during the same period from control patients (with uncomplicated gonorrhoea) of the same sex and from the same city (table V). Statistical analysis showed that disseminated gonococcal infection was significantly associated with both the AHU⁻ auxotype and with the PrIA-1 serovar ($p<0·05$).

PENICILLINASE PRODUCING STRAINS OF *N GONORRHOEAE*

Among all isolates from the three study groups, we identified 12 penicillinase producing *N gonorrhoeae*

TABLE III *Incidence of strains of Neisseria gonorrhoeae requiring arginine, hypoxanthine, and uracil and with PrIA-1 serovar from men with uncomplicated urethritis and from non-prostitutes and prostitutes with uncomplicated cervical infection in Lübeck in 1976-8 and 1980-2*

	No (%) positive/No tested in:	
	1976-8	1980-2
Men with uncomplicated urethritis	17/40(43)*	8/66(12)*
Non-prostitutes with uncomplicated cervical infection	8/13(62)	6/12(50)
Prostitutes with uncomplicated cervical infection	13/22(59)*	2/13(15)*

* = $p<0.05$

TABLE IV *Incidence of strains of Neisseria gonorrhoeae requiring proline and with PrIB-1 serovar from men with uncomplicated urethritis and non-prostitutes and prostitutes with uncomplicated cervical infection in Lubeck in 1976–8 and 1980–2*

	No (%) of positive/No tested in:	
	1976-8	1980-2
Men with uncomplicated urethritis	0/40*	11/66(17)*
Non-prostitutes with uncomplicated cervical infection	0/13	2/12(17)
Prostitutes with uncomplicated cervical infection	0/22	1/13 (8)

* = $p<0.05$

(PPNG) strains in 1980-2. Apart from clusters of three prototrophic/PrIA-1 and three prototrophic/PrIB-5 strains, each of the other six strains represented a different auxotype and serovar class (table VI). The strains in each of the clusters were epidemiologically related: the three prototrophic/PrIA-1 strains were isolated from three different male contacts of a prostitute in Northern Germany; and the three prototrophic/PrIB-5 strains were isolated from a prostitute and two of her male contacts in Heidelberg.

ANALYSIS OF PAIRED ISOLATES FROM MULTIPLE ANATOMICAL SITES

Of the 389 strains of *N gonorrhoeae* in our study, 46 were pairs of isolates from two different anatomical sites from 23 patients. Seventeen patients yielded urethral and cervical isolates, three yielded urethral and rectal isolates, two yielded urethral and throat isolates, and one yielded isolates from the urethra and Bartholin's gland on the same day. In 20 (87%) of the 23 instances, paired isolates were of the same auxotype and serovar. The three pairs of non-matched isolates were from the urethra and cervix of three women patients, two of whom were prostitutes. All three pairs had different serovars, and two pairs also had different auxotypes.

Discussion

Few epidemiological studies of infections with *N gonorrhoeae* have been made in the FRG. In 1979 Petzoldt *et al* described increasing resistance of gonococci to penicillin during the previous 22 years.[8] Bojanowsky *et al* found that in Mannheim prototrophic strains of *N gonorrhoeae* were characterised by increased resistance to penicillin and increased leucotactic activity.[9] No studies have subsequently been performed using modern serological methods to classify *N gonorrhoeae*.

In this study, we showed an appreciable change in the gonococcal population in Lübeck during a relatively short time spanning about four years. Dual classification by auxotype and serovar showed that this change included a decrease of $AHU^-/PrIA^-1$ strains and the emergence of $Pro^-/PrIB^-1$ strains.

A similar change in the gonococcal population has been described by Knapp *et al* in Copenhagen.[10] The AHU^- auxotype was detected in Copenhagen first in 1946, increased in prevalence to account for about half of all isolates in 1973-4, after which its incidence declined. In nine American cities, the proportion of AHU^- gonococcal isolates ranged from 6% to 57% of isolates, and tended to be highest in cities with the highest proportion of white citizens.[11] In Kentucky before 1978, the AHU^- auxotype was predominant in white patients. After 1978 the percentage of AHU^- isolates decreased in white patients. The change in the gonococcal population in Kentucky was associated with introduction of the PAU^- auxotype, which was isolated more commonly from white than from black patients, and which was appreciably more resistant to penicillin than were gonococci of the other auxotypes encountered.[12] Factors responsible for changes in the prevalent strains of gonococci in various populations at different times remain to be defined.

In the study published here strains of *N gonorrhoeae*

TABLE V *Incidence of strains of Neisseria gonorrhoeae requiring arginine, hypoxanthine, and uracil and with PrIA-1 serovar from patients with disseminated gonococcal infection and from a matched control group with uncomplicated gonorrhoea*

	No(%) positive
Patients with disseminated gonococcal infection (n=7)	6(86)*
Controls (n=49)	20(41)*

* = $p<0.05$

TABLE VI *Classification by serovar and auxotype of penicillinase producing Neisseria gonorrhoeae (PPNG) strains isolated in the Federal Republic of Germany*

	Auxotypes	
Serovar	*Prototrophic*	*Proline requiring*
PrIA-1	3	
PrIA-4	1	
PrIA-6		1
PrIB-1		1
PrIB-3		1
PrIB-4		1
PrIB-5	3	1
Total	7	5

isolated from patients with disseminated gonococcal infection in the FRG were clearly associated with the AHU$^-$ auxotype and with the PrIA-1 serovar. Previous epidemiological studies of *N gonorrhoeae* indicated associations of AHU$^-$ strains with disseminated gonococcal infection,[13] with resistance to the bactericidal activity of normal human serum,[14] with high susceptibility to penicillin G,[15] and with asymptomatic urethritis in men.[16] The association of AHU$^-$ auxotypes with disseminated gonococcal infection was first described for isolates from Seattle,[13] and has been confirmed by workers from other locations.[17 18 19] Regional variations in the prevalence of AHU$^-$ auxotypes may correlate with regional variations in the incidence of disseminated gonococcal infection. Noble *et al* showed that the incidence of disseminated gonococcal infection correlated with the presence of AHU$^-$ auxotype of *N gonorrhoeae* in Fayette County, Kentucky.[20]

The 12 PPNG strains in our study belonged to eight different auxotype and serovar classes. In addition to three prototrophic/PrIA-1, and three prototrophic/PrIB-5 strains, each of the six other auxotype and serovar classes was represented by only one single PPNG isolate. None of the serovars were unusual in the FRG. Bygdeman *et al* found 11 PPNG strains in 849 consecutive gonococcal isolates from five Scandinavian towns.[21] Ten of these 11 ß lactamase producing gonococcal strains belonged to serovars rarely encountered in non-PPNG strains. In a separate study of 22 PPNG strains collected in Heidelberg in 1979-84 seven different auxotype and serovar classes were detected. Four (18%) out of the 22 strains belonged to the Pro$^-$/PrIB-4 class, 10 (45%) belonged to the prototrophic/PrIB-5 class, and no rarely encountered serovars were detected (H Hofmann, N Dickgiesser, U Pekar, P K Kohl, D Petzoldt, unpublished data).

The diversity of the auxotype and serovar classes of PPNG strains showed that PPNG strains in the FRG had multiple sources and did not represent the introduction and spread of a single PPNG strain. Handsfield *et al* showed that the spread of a single imported strain was responsible for an outbreak of infection in Shreveport, United States of America.[22] In contrast, outbreaks in Washington State resulted from several different imported PPNG strains.[22]

The study published here confirms the association of AHU$^-$ auxotype with the PrIA-1 serovar.[6] Though Pro$^-$ auxotypes were associated with six PrIA and nine PrIB serovars, the requirement of proline was associated most strongly with the PrIB-1 serovar in this study, whereas PAU$^-$ auxotypes were appreciably associated with the PrIB-2, and PrIB-16 serovars. It remains to be seen whether the latter two correlations will be found elsewhere.

In a worldwide collection of 1433 strains PrIA-1 and PrIA-2 serovars accounted for 143 (98%) of 146 AHU$^-$ auxotypes, and only 32 (11%) of 287 WI serogroups belonged to all other auxotypes; no other correlation of auxotype with serovar was apparent.[6]

In the study published here we detected nine of the 18 known PrIA serovars, 14 of the 28 known PrIB serovars, and 48 of the 107 previously described auxotype and serovar categories. Despite the close proximity of Lübeck to Scandinavia, we were not able to detect any of the serovars that have been described previously only in Sweden.[21]

Our study shows that, despite the association of some auxotypes with certain serovars, the combination of serological classification with auxotyping permits very detailed analysis of populations of *N gonorrhoeae* because greater discrimination is possible than when using either method alone. Strains that seemed to be identical by one method can now be distinguished by the application of both classification systems. This increased accuracy of resolution makes it possible to trace temporal changes in gonococcal populations and to gain further insight into the epidemiology of gonorrhoea.

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